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I. The Red Tides around our coastline are due mainly to blooms of Dinoflagellates. Those in the colder northern waters are due mainly to the Gonyaulax sp. which produce the Paralyte Shellfish Poison Saxitoxin which is a Neurotoxin. In the waters of the Gulf of Mexico off Florida, the Red Tides are due to the Gymnodinium breve which kill millions of fish during a bloom. → next page

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cont. → Two major compounds

~~As a direct result of support under the O.N.R. contract we~~ have been separated by chromatography and have identified ~~two major compounds which are~~ as biologically active. One of these compounds has been identified ~~by Dr. Rappoport~~ as a pure compound which he has called Gymbretoxin, and which we have shown to be a potent neurotoxin. The second compound of small molecular weight is a hemolytic agent.

We have shown that the Gymbretoxin acts specifically on excitable membranes on the site of the Active Sodium Channels. The mode of action is by competition with the calcium ion on the resting membrane moving the α (Na inactivation) curve along the axis of membrane potential, and lowering the accommodation level.

Martin and Padilla have shown that our second compound has a hemolytic actin on fish red blood cells which is far more intensive than on mammalian red blood cells.

We have investigated in fish tissues the influence of the various components. Small mosquito fish (Gambusia afinis) were placed in finger bowls. Three sets of studies were carried out.

1. fish were killed by exposure to the crude muscle toxin after only one extraction step.
2. fish were killed by exposure to pure Gymbretoxin at a level of 10 FTU/ml.
3. fish were exposed to the hemolytic fraction at an equivalent concentration to experiment (2). The experiment was terminated after 15 minutes without death and the fish then killed.

The dead fish in each experiment were dissected and selected tissue studied for cytolysis.

Paralled experiments were carried out to study the hemolysis resulting from exposure of red cells to each of the three situations above.

The results showed clearly that the crude toxin produced death with no evidence of cytolysis, although direct exposure of red cells to the crude toxin caused hemolysis. The pure toxin Gymbretoxin produced neither cytolysis nor hemolysis. The pure second fraction which hemolysed red blood cells also produced cytolysis of fish muscle cells ~~although it did not kill the fish in the duration of the experiment.~~

II. The remainder and major emphasis of the research under the contract has been devoted to the occurrence of Red Tides in the Los Angeles-Long Beach Harbor complex. The roles and relevance of a number of ambient parameters have been studied, and the influence of polluting nutrient outflows have been evaluated. ←

III. An extension of the contract without funding enabled a final histological study of the action of the Gymbretoxin to be made. The hemolytic factor produced significant hemolysis, but the neurotoxin showed no evidence of pathological changes.

Marine Pollution Hazards

Office of Naval Research

Final Report, N00014-67-A-0269-0017

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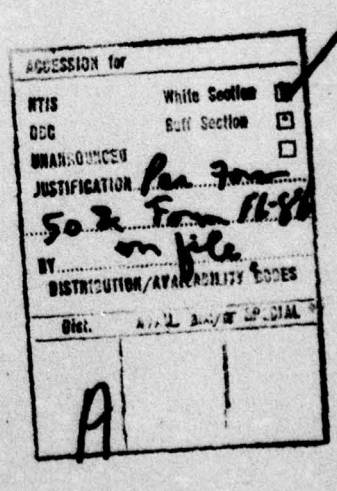

BACKGROUND

This program of research has been devoted to the study of many aspects of the dinoflagellate blooms which constitute the Red Tides of the coastal waters. Dinoflagellate cells are always present in the ocean. Only occasionally are the conditions adequate for bloom conditions. Biotic and abiotic factors, at such times, combine to produce dense concentrations of the cells in limited areas. The precise combinations of external parameters are still unknown. Certainly, as the urban developments of our coastal zone have multiplied, and the organic waste effluents have increased, so the occurrence of Red Tides have increased. We have studied the effect of some effluents on growth patterns, emphasizing, in particular, the effects of wastes likely to be derived from Naval ships in harbor. The cells photosynthesize so that in daylight the oxygen levels are high. Only as the bloom dies off, and organic compounds are released, does the high BOD induce anaerobic conditions. Then animals are killed and physical damages mount. Toxicity, however, can arise from the cells themselves. All cells produce metabolites, but certain dinoflagellates produce metabolites which are poisonous.

In the case of Gymnodinium breve, we have purified an intracellular hemolytic component and a neurotoxin, whose mode of action has been studied in detail.

PERSONNEL

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RESULTS

The studies carried out on this contract have included studies, in the main, on two species of Dinoflagellates. The first is Gymnodinium breve, and the major interest has been devoted to the purification of ichthyotoxin produced by the cells. The second species, Gonyaulax polyhedra, is the causative organism in most of the Red Tides of Los Angeles Harbor, and the studies have emphasized the Biotic-Abiotic factors of harbor productivity.

1. Gymnodinium breve. These organisms are single celled naked dinoflagellates, and form the essential causative organism of the Red Tides off the Gulf Coast of Florida. These Red Tides are characterized by extensive fish kills, and our studies have concentrated on the identification and mode of action of this ichthyotoxin.

a. Growth Studies. In order to study the toxin produced by cells in culture, it is essential that the culture be unialgal and also free of bacteria. And, in order to obtain adequate supplies of the toxins, mass cultures are needed: each cell has a diameter of only about 15 u, and the contents of a billion cells are needed for a microgram of pure toxin.

The elimination of bacteria from unialgal cultures of a breve, grown in defined media, was successfully accomplished with the use of the antibiotic kanamycin. When an aliquot was taken for subculture, the resultant growth was masked by a lag phase of up to 10 days. After the lag period, growth occurred at a normal rate (an average doubling time of about 4 days) and to concentrations of up to 40,000 per ml. This requires growth times of three to four weeks before harvesting. It was found that hormonal concentrations of Gibberellic Acid reduced the lag time from 10 days to only a few hours, so that harvesting could be made every two weeks. This was initiated as a practical procedure, but led to an investigation of the cause.

Studies followed two major directions. There is no clear indication of the cause for the presence of a lag phase. In general, the lag is least if cells are subcultured from the exponential phase of growth, and the lag increases the nearer the culture is to the limiting plateau phase. It has been suggested that during the lag phase of growth the cell metabolism is directed towards the synthesis of proteins and cell constituents, whereas, in lag phase, the metabolism is directed towards the manufacture of storage products, such as Glycolic Acid. In the logarithmic phase of growth, therefore, there

will be great activity of the RNA polymerase in order to produce the templates for protein production. Studies were therefore made of the RNA polymerase profiles of the G. breve cells. Using Isoelectric focusing techniques, we found many more bands present than the conventional three. The studies have led to a more detailed and separate investigation of the kinetics of RNA polymerase, but activation of certain bands by Gibberellic Acid was demonstrated.

Gibberellic Acid is a compound related to growth cycles in higher plants, and had not previously been demonstrated to influence phytoplankton. In evaluating its effect on the growth of cultures, it was found that after priming a culture with Gibberellic Acid, then subsequent subcultures in media free of the hormone, gave no lag phase for the first subculture, and progressively larger lags for subsequent ones. Thus, the hormone is apparently retained within daughter cells for some time. And, it was observed that in non-sterile unialgal cell cultures, the lag phase was minimal and Gibberellic Acid had no effect.

Because of the higher plant effect of Gibberellic Acid in inducing growth, attention was paid to another plant hormone, Abscisic Acid, which acts at the other end of the growth cycle and is responsible for the abscision of leaves and fruit.

The compound was very difficult to obtain, but a small amount was obtained. We found that in hormonal concentrations (10^{-7} M) Abscisic Acid stopped the growth of the cultures.

b. Extractions. The extraction techniques described in the previous report have been carried further and a pure ichthyotoxin has been isolated. This toxin has been labelled Gymbretoxin by Dr. Rappaport.

There is no doubt that even the crude toxin is lethal to all vertebrates if it enters the blood stream at above a certain very low concentration. Below that lethal threshold the effects are transitory. For example, in cats following intravenous injection of toxin, there is a rapid reduction in heart rate, accentuated respiration and muscle fibrillation which eventually die away. Intense irritation of human mouth tissues has been experienced in our laboratory when the dried powder of our extract T1 is transferred during weighing procedures, and Steidinger et al (1973) have provided a detailed account of the reactions by humans to exposure to the toxins. Similarly, mice exhibit considerable facial discomfort as indicated by pawing of the mouth if exposed to a weak spray of water which contains toxin.

Although the majority of studies have been made on culture extracts, concentration of toxic fractions occur when pelecypods are maintained in an environment containing G. breve cells. The molluscan tissues are poisonous if fed to chickens (Ray and Aldrich 1965) and appear, under the correct conditions, to produce a ciguatera like effect on test vertebrates (McFarren et al, 1965).

Our method of extraction has consisted essentially of a phase separation with a 2:1 chloroform methanol mixture against water. The organic phase is lyophilized, and the resultant crude powder is run on a Sephadex LH20 column with 2:1 chloroform methanol. Two biologically active components

could be separated (Spiegelstein et al 1973). The first one labelled GT in tubes 6-16 (5ml fractions) exhibited neurotoxic and lethal actions on fish. The second active component, tubes 33-63, exhibit hemolytic effects in vitro using rabbit erythrocytes but shows little or no neurotoxic activity. Purification by several groups of the neurotoxic fraction of the early tubes with higher molecular weight has produced different empirical formulae (Martin and Chatterjee 1970, Cummins and Stevens, 1970, Sasner et al, 1972). We have proceeded only to the stage of purification with TLC to use two neurotoxic fractions labelled T1 and T2 . These fractions are very toxic to vertebrates because even the cruder fraction GT is lethal to mice at 150 ng/g of animal. The mode of actions of the G. breve toxin has been investigated by Sasner (1973) who has presented reasonable evidence that his fraction IV has a depolarizing post synaptic action. His main evidence was obtained from a frog nerve-muscle preparation. Exposure of the junctional region produces fibrillation of the muscle followed by blockage of the junction as well as motor nerve and muscle if concentration of the toxin is high enough. The fibrillation effect can be blocked by curare.

We have made observations which offer another explanation. Our experiments were very similar to those of Sasner, also using a frog sartorius nerve muscle preparation. However, careful observation of the spontaneous movements which occurred when low doses of toxin were added to the fluid bathing region of the nerve muscle junction showed that the movements were fasciculations and not fibrillations. It was clear that numbers of fibers throughout the muscle were contracting synchronously. The effect could be

seen even on the mechanical records, and this suggests that individual Motor Units are contracting as entire entities. If the various fibers of a Motor Unit fire together, then the presynaptic endings must also be discharging simultaneously. In contemplating the implied firing of the motor nerve common to the motor unit family of fibers, we realized that a fifth alternative can be added to Sasner's four possibilities (1973, p. 160): spontaneous spikes in the nonmedullated termination of the motor nerve. Let us note the evidence:

1) 0.5 FTU/ml of our extract T1, after TLC was used in frog sartorius nerve-muscle studies. (Fish Toxic Unit, FTU, is defined in Spiegelstein et al, 1973 as that amount of toxin per ml which just kills 3 test fish in 3 hours under standard conditions).

2) At this concentration, spontaneous brief series of contractions occurred (fig. 1), but there was no loss of response to nerve stimulation.

3) The rise of tension in these spontaneous tetani could be blocked by curare.

4) No drop in the Resting Potential across the muscle membrane was detectable.

5) There was no change in the shape, amplitude or rate of the miniature endplate potentials (m.e.p.p.)

6) In the presence of 4 FTU/ml of extract T1 with 2.5×10^{-6} w/v curare, the mechanical responses were blocked, but trains of spontaneous End Plate Potentials (EPP) appear. These are seen in Fig. 2 recorded with intracellular electrodes. The traces run successively upwards with 0.5 sec sweep and 9 mv steps per sweep. Facilition can be seen in each burst and the beginning of depression in later bursts. The first burst shown lasted more than 10 sec. After a period of silence further bursts occur (Fig. 3).

An interval histogram (Fig. 4) shows that the impulses are not random. In the first burst there is a 10 msec interval which later (second and third histograms) shifts to a 20 msec interval together with higher multiples.

7) In our neurotoxic extract, we find no anticholinesterase activity as measured by the Jensen Holm method (1969) even at 400 times the mechanical threshold (Fig. 5).

We believe that this evidence supports the proposition that at low concentrations the extract T_1 produces bursts of spontaneous discharges in the terminal nonmyelinated region of the motor axon in a fashion similar to the effect of bathing the nerve in low Ca^{++} medium. This possibility was strongly supported by the demonstration that when the Ca^{++} in the Ringers solution was increased ten fold (to 10mM) the excitation action of the low level of toxin was immediately blocked.

Finally, a comparable action of the extract T_1 on squid axon has been demonstrated with the measurement of membrane potential by an intracellular electrode. It is known that the natural accommodation factor of axons vary widely between specimens, and this is recognized in the response to 4 TU/ml toxin. And indeed a variety of responses were observed from continuous discharges to small subthreshold oscillations which build up to a train of spikes and also spike trains of constant interval which trail off into damped oscillations (Fig. 6). There were also instances of very small subthreshold oscillations of the MP evoked by a brief pulse which died away without initiating a train. Such depolarizations of the membrane are very small and may be difficult to identify (Fig. 7).

Thus, we are suggesting that the toxin from G. breve acts to lower the level of the accommodation factor of excitable membranes. This can be expressed in terms of the extent of sodium inactivation which exists in the

resting state, and we suggest that when the membrane is exposed to toxin the percentage of sodium inactivation decreases without change of resting membrane potential. This decrease in activation will lower the threshold for excitation because less current flow is needed to produce a local response. As the threshold drops towards the resting membrane potential, the accommodation factor falls and spontaneous discharges may occur. This can also be expressed as a statement that the α curve of the Hodglian-Huxley theory is shifted several milliwatts in the directions of depolarization. Only those membranes in which accommodation is already low would be expected to reach the point of instability when toxin is added. It would appear that in vertebrates and certain invertebrates, the primary action of the T_1 fraction of G. breve toxin is to induce spontaneous discharges in nonmyelinated axon similar to the effect of low calcium. At high concentrations the myelinated nerve and the muscle membranes become inexcitable, and this may well be an extension of the same phenomenon. The effects on fish in an actual Red Tide produces disorganization of the central nervous system and death while the skeletal muscles and myelinated nerves are still excitable. The precise cause of death is not known, and studies are needed to see if our suggestion that bursts of discharges in the nonmedullated nerves actually occur. It can explain the slowing of heart rate through discharges of the vagal endings and the elimination of this effect by the addition of atropine.

2. Studies on the Red Tides of Los Angeles Harbor. The Red Tides in the Los Angeles Harbor can be composed of several dinoflagellates,

but the most common bloom is due to Gonyaulax polyhedra. An investigation has been undertaken of the biotic and abiotic influences present in the harbor, in order to determine some of the factors related to Red Tide occurrences. A narrative account is given of the total investigations carried out in the Los Angeles Harbor, of which this contract constituted an integral part.

The Los Angeles-Long Beach Harbors had never had a coordinated biological, physical and chemical survey until the initiation of the present studies by Harbor Environmental Projects of the Allan Hancock Foundation, University of Southern California, in spite of the fact that it is one of the major ports in the United States and houses a Navy base. Under the impetus of the National Environmental Policy Act of 1969 and subsequent legislation, private industry and public agencies found a need for environmental information that either did not exist or was proprietary and hence not available to them. Accordingly, a baseline survey and monitoring program was begun by the University for the outer Los Angeles Harbor in 1971, under sponsorship of Pacific Lighting Service Corporation, and expanded in 1972 under the USC Sea Grant Program (Department of Commerce - NOAA) and contracts with the Los Angeles Board of Harbor Commissioners. The U. S. Army Corps of Engineers, Los Angeles District, in 1973 contracted to extend the program to cover the entire harbor, and added to the list of parameters to be studied. These investigations are now completed and computer analysis of the data has been carried out.

A total of 43 stations was selected to be monitored for this study (Figure 1). The locations selected were chosen to permit the optimal coverage of areas and

expected patterns of variability within the scope of our facilities. These stations were divided into regional groups for convenience in dealing with the operational time and work load constraints involved in the program of sampling and data analysis.

Monthly sampling at each station included remote sensor measurement of water temperature, salinity, pH, dissolved oxygen and turbidity at one meter depth intervals through the water column. Surface water samples were collected for analysis of dissolved oxygen (DO) by Winkler titration and for determination of BOD, NO_3-N, NO_2-N, NH_3-N, PO_4-P, and sulfide. Biological sampling included collection of surface water for determination of bacterial populations including coliforms, phytoplankton productivity and chlorophyll a. Also surface tows were made for zooplankton, using a 253 micron mesh 1/2 meter net equipped with a flowmeter. Screened settling racks containing glass slides were deployed monthly at 24 stations throughout the harbor to sample water column fauna. Separate sampling operations were conducted for determining fish crop and for bird census.

At approximately quarterly intervals each station was sampled for determination of sediment grain size and for benthic fauna, using either a modified Reinecke box corer or a small Campbell grab. Chemical analysis of sediments for 33 parameters of trace and heavy metals and chlorinated pesticides were performed at 43 stations within the harbor. Under separate contracts with the Los Angeles Harbor Department sediment chemistry was determined for 60 stations in 4 transects across the San Pedro Channel to Santa Catalina Island, for

comparison with pollutant levels in the harbor. Bioassay and toxicity tests on harbor benthic, planktonic and pelagic species were also carried out to determine potential effects of proposed massive dredge and fill operations for port development. Thermal effluents and toxic wastes were similarly tested with bioassay techniques to determine ranges of tolerance and identify interference with reproductive capabilities.

The Los Angeles and Long Beach Harbors are actually part of a single water area which has been artificially restructured by dredging from the river mouth sediments and channels of the Los Angeles River basin and adjacent waters of San Pedro Bay. Terminal Island is primarily constructed of fill. The harbor is divided roughly in half by a dotted line of competing local jurisdictions, the Ports of the two cities. While water masses, pollutants and organisms do not recognize these boundaries, local, regional, and national public agencies control some or most of the activities within the water and the peripheral land area.

Southern California is a young area geologically, and several times during Pliocene and Pleistocene periods sea levels varied so that the entire basin was submerged. At other periods of receding waters, the Channel Islands may have been connected to the mainland. The Los Angeles River bed changed course several times, exiting into Santa Monica Bay to the west for some periods, and most recently to the south in an estuarine configuration. Further eastward, the San Gabriel River also empties to the south, but both rivers were diverted to permanent channels some forty years ago. Thus the watershed of the San

Gabriel mountains 30 miles to the north of the basin drains into areas southeast of the two ports. Although estuarine in configuration, the area cannot be classified as a true estuary, with continual freshwater flow. Instead it receives an annual rainfall of 9-14 inches entirely in the winter months. Sewer outfalls provide the only other major freshwater flow, and that mixes in the outer harbor sufficiently that biological gradients due to salinity differences cannot ordinarily be seen.

Evidence from our studies indicates that the Los Angeles River channel is an unexpected major source of pollutants, apparently because catch basins upstream receive debris and waste drainage which is carried downstream during major storms. In January, 1974, 9 inches of rain fell in a single storm of several days' duration, and affected the annual mean levels of pollutants, as mapped by computer. The oil well islands, to the southeast of the river, are outside the harbor jurisdictions, but evidence was accumulated that they may be intermittent sources of heavy COD. The San Gabriel River mouth carries large thermal effluents which do not seem to exert any extensive deleterious effects on biota.

The principal problems facing both ports, in addition to the multiplicity of jurisdictions, rise from the need to modernize port facilities and deepen channels for containerized cargo and for receipt of Alaskan and Indonesian oil and liquified natural gas (LNG). On the west, in Los Angeles Harbor, the main channel depth is only 35 feet, due to the underlying bedrock of the Palos Verdes limestones. To the east, the Long Beach Harbor channels range from 35 feet inside to about 80 feet at the entrance. Much of their land is "fill," and subsidence has occurred where oil drilling was carried out on Terminal Island. Inner harbor channels are

narrow, and much land is owned by railroad and oil companies so it is not available to the Port for general cargo. Hence the ports plan extensive dredging and building of new land in the outer harbor utilizing underwater diking and treatment of dredged sediments with polymers in order to contain pollutants as inert fill.

The California Environmental Quality Act requires preparation of Environmental Impact Reports (EIR) in order to predict the impact of various proposed changes on the coastal waters which might affect the environment and urban growth. To implement port modernization, the basic biological, chemical and physical marine studies are needed. As these were performed during our studies, alternate sites for port facilities were also examined. The large team of investigators agreed in general that it would be better to impact an area already extensively influenced and modified by restructuring and by pollutants than to destroy the few relatively natural estuarine areas remaining in southern California. Chief among these natural areas are Morro Bay and Mugu Lagoon to the north, and Anaheim Bay on the southeast. The latter two were contained within naval bases for years and the natural environment preserved, although their interior watersheds were altered by adjacent urbanization.

The following sections of the present paper discuss briefly some of the findings of these studies, which form a baseline for determining future impact. A scientific journal, Marine Studies of San Pedro Bay, California, was established to make readily available the data and information that are needed by the southern California community and by public agencies. To date, nine volumes have been published by the Allan Hancock Foundation and the USC Sea Grant Program. A

summary report has also been circulated by the U.S. Army Corps of Engineers, who are responsible for navigable waterways and for issuing permits to dredge, dump and otherwise alter those waterways.

Tidally generated water circulation in outer San Pedro Bay is dominated by two large patterns, separated by Pier J in Long Beach Harbor. To the west, water enters and leaves Angels Gate on a rising tide and feeds a large gyre in the outer basin. A smaller gyre to the west off Carbillo Beach appears intermittently and a portion of the water enters and leaves the inner harbor by the main channel. Most of the water entering Long Beach main channel is also derived from the gyre.

The area east of Pier J is fed by the Los Angeles River and by water entering Queens Gate on a rising tide and forms a series of minor complex gyres, with water also entering from the east. The net water movement, however, is eastward. On a falling tide the water leaves the harbor through the eastern breakwater opening with a small portion flowing out of Queens Gate.

The described patterns indicate that two, almost separate, hydrodynamic systems are responsible for distribution of various parameters in the study area in the harbors of San Pedro Bay.

Inspection of the results for this study shows fairly uniform annual temperature means except in the vicinity of the Alamitos generating station cooling water outfall at the San Gabriel River mouth. Here temperatures are elevated considerably over those prevailing in other areas.

Mean salinity at the various stations point out four major sources of fresh water entering the harbor. Low salinities at the Cabrillo Beach area and the West Channel probably are due to runoff and impoundment, since water circulation is somewhat restricted there. The area east of Fish Harbor is the location of two

cannery waste outfalls and one large municipal sewage effluent line. Water discharged there enters the large current gyre in the outer harbor and is therefore primarily retained in the harbor, causing some small reductions of salinity levels throughout the outer harbor. The sewage presently receives only advanced primary treatment, so that the outer harbor acts as a natural oxidation pond. The Los Angeles River carries runoff and assorted waste discharges from inland. The area of the Alamitos generating station outfall at the San Gabriel River mouth is the last of the four sources of fresh water in the harbor. Tidal flushing patterns were significantly altered when the Pier J fill to the east was constructed by Long Beach in the 1940's. Previously the tidal flow entered the eastern channel, Queens Gate, slightly earlier than at the westward Angels Gate. The nodal point occurred near the lift bridge in the middle of the inner channel, and surge damage occasionally occurred there. After the Pier J fill, the easterly tidal flow was diverted to the east and north, and served to flush, or mix with, river effluent along the shore. Beach sampling showed that area to be impoverished biologically.

The easterly circulation pattern gyres may help to create "water cells" in the area which are not adequately flushed and may help to create the red tide dinoflagellate blooms which occur periodically in the harbor and adjacent waters.

Nutrient chemistry measurements revealed that there are two major sources of phosphate (PO_4-P) in the study area; the waste outfalls area, and the San Gabriel River mouth area.

The major source of nitrite (NO_2-N) was the Los Angeles River mouth and two minor sources were at the outfalls area and at the San Gabriel River mouth.

The major nitrate (NO_3-N) concentration occurred at Cabrillo Beach, a public recreation area with beach, fishing pier and small boat facilities, while the river mouths showed minor concentrations.

The waste outfalls showed low values of NO_3-N but the highest values of ammonia (NH_4-N). Ammonia was also high at the river mouth.

High nutrient concentrations in the harbor and bay appear to be associated with the areas of freshwater input and lowered salinities; this suggests a terrigenous origin for the nutrients, or possibly multiple origins from waste effluents and land run-off.

Mean dissolved oxygen (DO) levels were lower in the four areas of lowered salinities, while higher values appeared in the areas of the main gyre systems; in the outer Los Angeles Harbor, and between the two river mouths (Figures 2,3).

The pattern for high biochemical oxygen demand (BOD) coincided most closely with the pattern for ammonia, namely, highest at the waste outfalls and next highest at the two river mouths. The inverse occurrence of DO and BOD levels demonstrates the heavy organic loading of effluent waters and run-off in those areas.

Microbial surveys of presumptive total coliforms, fecal coliforms and fecal strep and Standard Plate Counts were carried out. Standard Methods utilizing distilled water for BOD and microbial counts were compared with methods developed to use seawater as a diluent (Juge and Griest, 1975). This suggests that the usual public health sampling methods may not show the extensive persistence of coliforms in sea water. Counts were high, as might then be expected in

the outfalls area, and in the gyre. High counts at the Los Angeles River coincided with the 1974 heavy rain storm. High total coliform counts, without confirming fecal coliforms, indicate a terrigenous rather than a sewage origin. There is some controversy as to whether coliforms in fish guts are normal, or indicate ingestion of contaminated waters or wastes.

While the high microbial levels could be considered as a public health problem, no instances of human illness have been documented from the area. The outfalls area is posted against fishing and water contact sports, but local residents can be seen along the jetties fishing, nevertheless.

The fact remains that the harbor fish standing crop is the highest of any local area, inside or outside the harbor (Stephens et al., 1973, 1974; Chamberlain, 1973, 1974).

Radioactive tagging of amino acids in cannery wastes by Chamberlain (1975) showed that the wastes could be directly taken up by fish without microbial recycling or an intermediate food chain. While a zone of inhibition of biological life occurred at the immediate site of the outfalls, the areas adjacent to them, near or in the better oxygenated gyre, showed exceptional crops of benthic and planktonic organisms.

Phytoplankton productivity measurements using ^{14}C inoculation and incubation showed moderate values in the outer harbor areas. To the east, a gradient occurred, with highest values occurring near the Los Angeles River mouth, decreasing in an easterly direction toward the San Gabriel River mouth. This pattern was duplicated in the chlorophyll a values. Assimilation ratio values

were moderately high near the outfalls and in the outer harbor areas. The gradients of productivity and chlorophyll a values were reversed in the area to the east for assimilation ratios; values were low at the Los Angeles River and higher toward the San Gabriel River.

Zooplankton densities calculated as settling volumes were roughly in agreement with the gradient of phytoplankton assimilation ratios. Wet weights of fauna collected on settling racks were also determined. Patterns of distribution for holoplankton and meroplankton showed decided preferences for different areas by different species.

The investigation of physical, chemical and biological parameters, within the scope of a single baseline study and ancillary research, permits the application of computer analysis techniques; hence correlations, or the lack thereof, are revealed which might not otherwise be apparent under subjective evaluation.

Selection of the mode of analysis can, of course, influence the patterns displayed. In general, means were utilized and extremes were transformed and standardized so that basic trends rather than variability could be seen. Hence, for example, the most common species are used in dendrogram analysis, and the extent of diversity is only revealed in the computer lists.

The patterns for the biotic and abiotic parameters are clearly demonstrated by the methods for computer mapping of data, developed by John McDonald, a University of Southern California geographer. Computer classification and ordination programs were developed by Robert W. Smith, of the Allan Hancock Foundation, for analysis of biotic-abiotic relationships.

The most consistent general pattern for species distribution and related physical parameters divides the harbor into three area types: 1) Polluted innermost slips with piling communities and low circulation; 2) main channel fauna where circulation is better; and 3) the outer harbor with extensive fauna, high nutrients, and the best circulation. Since the current outside the harbor is probably not more than one half knot and tidal flushing is reduced, circulation even in the outer harbor is not particularly good.

It seems clear that plans to cut down extensively on water areas in the outer harbor and put in slips and piers will alter the species composition of the biota to a piling or fouling community.

Since the harbor sediments are grossly contaminated (Chen and Lu, 1974) hydraulic dredging and filling, followed by treatment with polymers should reduce that pollution. Hydraulic dredging can be carried on with a very minimal sediment plume, but vast numbers of benthic polychaetes will be eliminated by fill. Experiments in the resuspension of contaminated sediments carried out in our laboratories have shown that chemical changes occur in the pollutants, which are absorbed on fine silt and clay particles and may be inert in that form. Certain of the planktonic and benthic crustaceans were particularly vulnerable. Standard toxicity tests which require only 96 hour exposure to a 1:4 ratio of sediment to sea water, have often showed no impact, whereas longer term tests which include the ability to survive and to reporduce have indicated a reduced reproductive capacity.

Although the Los Angeles Harbor was virtually dead prior to the

enforcement of water quality standards in 1969-70, an extensive fauna has developed since that time. The most important single action was probably diverting of oil field and refinery wastes into sumps. Gradually the water quality has improved, to the point where no areas are barren of life and some areas are rich in both diversity and biomass.

It will be very difficult to protect these gains in the proposed new port configurations. The probable use of the port as a major oil terminal and trans-shipping point also suggests that constant care will be required to prevent accidental spills. This problem is controlled well in many ports by complex computerized controls and double safety systems.

Spills from ship collisions will no doubt continue due to human error, but containment and clean-up techniques have improved greatly. While monobuoys have been widely proposed for supertankers outside of harbors, the problems of containment are greater there. One problem that affects the biota on a world-wide scale is the tanker practice of blowing tanks while at sea. Even isolated coral reefs far from harbors but on shipping lanes show globs of crude in festoons on the corals. It will take particular care and vigilance to preserve some biological diversity and maintain the precarious biotic-abiotic balance presently achieved in the Los Angeles-Long Beach Harbors.

3. Supplementary Report of Extension of Contract. We have shown that the Gymbretoxin acts specifically on excitable membranes on the site of the Active Sodium Channels. The mode of action is by competition with the calcium ion on the resting membrane moving the α (Na inactivation) curve along

the axis of membrane potential, and lowering the accommodation level.

Martin and Padilla have shown that our second compound has a hemolytic actin on fish red blood cells which is far more intensive than on mammalian red blood cells.

We have investigated in fish tissues the influence of the various components. Small mosquito fish (Gambusia afinis) were placed in finger bowls. Three sets of studies were carried out.

1. fish were killed by exposure to the crude muscle toxin after only one extraction step.

2. fish were killed by exposure to pure Gymbretoxin at a level of 10 FTU/ml.

3. fish were exposed to the hemolytic fraction at an equivalent concentration to experiment (2). The experiment was terminated after 15 minutes without death and the fish then killed.

The dead fish in each experiment were dissected and selected tissue studied for cytolysis.

Paralled experiments were carried out to study the hemolysis resulting from exposure of red cells to each of the three situations above.

The results showed clearly that the crude toxin produced death with no evidence of cytolysis, although direct exposure of red cells to the crude toxin caused hemolysis. The pure toxin Gymbretoxin produced neither cytolysis nor hemolysis. The pure second fraction which hemolysed red blood cells also produced cytolysis of fish muscle cells although it did not kill the fish in the duration of the experiment.